

AD _____

Award Number: DAMD17-01-1-0244

TITLE: The Establishment of an Inflammatory Breast Cancer
Registry and Biospecimen Repository

PRINCIPAL INVESTIGATOR: Paul H. Levine, M.D.

CONTRACTING ORGANIZATION: The George Washington University
Washington, DC 20052

REPORT DATE: August 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20050516 048

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE August 2004	3. REPORT TYPE AND DATES COVERED Annual (1 Aug 2003 - 31 Jul 2004)	
4. TITLE AND SUBTITLE The Establishment of an Inflammatory Breast Cancer Registry and Biospecimen Repository			5. FUNDING NUMBERS DAMD17-01-1-0244	
6. AUTHOR(S) Paul H. Levine, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The George Washington University Washington, DC 20052 E-Mail: sphphl@gwumc.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) The Inflammatory Breast Cancer (IBC) Registry enrolled its first patient Sept. 10, 2002. As of July 31, 2004, 163 patients have contacted the Registry and 135 have completed their interviews. Tissue blocks have been obtained from 110 patients and frozen surgical specimens have been collected from 10. Five laboratories were sent biospecimens and results continue to be obtained. Data on the first 50 patients were presented at the San Antonio Breast Cancer Conference in Dec. 2003 and initial evaluation of the questionnaire data will be presented at the San Antonio Breast Cancer Conference in December, 2004. The clinical data include the observation that approximately one third of IBC patients are initially diagnosed as having mastitis and are treated with up to five months of antibiotics before the diagnosis of cancer is made. Less than 25% of patients have a discrete mass identified on initial mammography. Laboratory data thus far (45 patients) indicate that the tumors from all patients express significant amounts of BP-1, a homeobox gene associated with breast cancer aggressiveness. In addition, tumors from a higher percentage of IBC patients express gene sequences resembling mouse mammary tumor virus than non-IBC breast cancer patients.				
14. SUBJECT TERMS Inflammatory breast cancer registry			15. NUMBER OF PAGES 8	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	
SF 298.....	
Table of Contents.....	1
Introduction.....	2
Body.....	2
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	6

INTRODUCTION

Studying inflammatory breast cancer (IBC), the most aggressive form of breast cancer, may provide an understanding of aggressive breast cancer and the biology of breast cancer in general. Since IBC is relatively rare, we have developed a national registry of patients with IBC which contains standardized clinical, epidemiological and pathological information. Our registry includes both the clinical classification (redness, warmth, and edema) and the pathological classification (invasion of the dermal lymphatics). By standardizing clinical and pathologic information, we have an excellent opportunity to investigate the heterogeneity of IBC. We are characterizing the tumors of the IBC patients by using a panel of biomarkers through the implementation of a biospecimen repository. The specimens we collect include formalin fixed material (stained and unstained) and frozen tissue (normal and tumor). New technological advancements in molecular biology have made it possible to study biomarkers in these tumors. The specimens are needed more than ever to provide opportunities for critical translational research focusing on the pathogenesis of breast malignancies. We have sent biospecimens to five laboratories and continue to receive results for evaluation. We also make our specimens available to qualified investigators for new studies to facilitate their research. This registry will serve as a source of useful epidemiological data for investigators who are studying IBC and can be used to generate hypotheses that might be tested in subsequent epidemiological studies.

BODY

The purposes of this project are: 1) to develop a well-documented Registry of patients with IBC, 2) to establish a bank of biospecimens and 3) improve the diagnostic criteria for IBC. The repository will be made available to researchers who are doing research on the etiology and pathogenesis of IBC.

Tasks (objectives of project)

1. To identify patients with IBC who are willing to provide relevant information.

We have developed close communication with two Web-based IBC support groups that inform patients how to contact the IBC registry. As of July 31, 2004, 163 patients have contacted the IBCR.

2. To develop a questionnaire to obtain epidemiological information on IBC patients. The questionnaire is based on findings from previous studies on IBC and aggressive breast cancer and other reports of relevant factors.

The questionnaire has been completed and 135 women have been interviewed to date. The principal investigator also interviews each patient to gather clinical information which helps to classify each patient according to category (see Table 1).

Table 1: Case Categories

- Group 1: Classical history and physical findings, pathological confirmation
- Group 2: Classical history and physical findings, no pathological confirmation
- Group 3: Incomplete clinical findings of IBC, pathological confirmation
- Group 4: Incomplete clinical findings of IBC, no pathological confirmation
- Group 5: Pathologic findings without clinical features
- Group 6: Secondary IBC
- Group 7: IBC vs. neglected breast cancer
- Group 8: Apparent neglected breast cancer

3. To obtain paraffin blocks, and when feasible, freshly frozen tissues to establish a biospecimen repository.

Tissue blocks have been obtained from 110 patients and frozen surgical specimens have been collected from 10. A Biospecimen Advisory Board was established and procedures are now in place to send biospecimens to requesting laboratories on a pilot basis and determine the number of subsequent specimens to be sent based on the initial results. Biospecimens have been sent to five laboratories and data are now being received.

4. To collect and enter into a database information from the questionnaire, information on recurrence and survival, clinical and pathological information, and information on the presence of biomarkers.

Two password protected access databases have been created to store data from the questionnaire and from the principal investigator's interview.

5. To make the biospecimen repository available to researchers.

The Principal Investigator, Dr. Paul Levine, has presented the project and the availability of biospecimens to researchers at the 2002 San Antonio Cancer Conference in San Antonio and again described the availability at a presentation in the 2003 San Antonio Breast Cancer. All publications involving the IBC Registry will include this information.

Current findings

1. Questionnaire analysis

Since the last report, a major focus has been the comparison of questionnaire data on IBC patients with non-IBC breast cancer patients. As of July 31, 2004, 324 women from GWUMC with non-inflammatory breast cancer have been interviewed and data have been analyzed on 215. Tumor aggressiveness in the non-IBC patients was defined by tumor grade, with those patients having Grade 1 and 2 tumors being classified as non-aggressive breast cancer and those with grades 3 and 4 classified as aggressive breast cancer.

The most important finding was that early age at first birth was the most significant factor that

correlated with tumor aggressiveness. Analyzing the non-inflammatory breast cancer patients, 76% of the 34 women who had their first child before age 20 had an aggressive tumor. Of the 85 women with aggressive breast cancer who had children, 26 (31%) had their first child before age 20; of the 69 women with non-aggressive breast cancer who had children, 8 (9%) had their first child before age 20. Of the 98 inflammatory breast cancer patients with children, 15 (15%) had their first child before age 20. Long term hormonal therapy was significantly associated with aggressive breast cancer in the non-inflammatory breast cancer patients with aggressive disease vs. those with non-aggressive disease. The data for IBC patients are currently under analysis and the results will be available for presentation at the meeting. The comparison of risk factors for tumor aggressiveness in patients with IBC vs. those with aggressive non-IBC may be important in determining if there are unique factors contributing to the etiology of IBC. Thus far, early age at first birth, significant in aggressive non-IBC patients, does not appear to be as strong a risk factor for IBC. We are currently investigating other risk factors, such as breast trauma and the onset of breast cancer in the peri-gestational period as being significant contributions to the etiology of IBC.

2. Laboratory Studies

Among the important laboratory findings are the identification of BP1 immunoreactivity in all cases of IBC with intensities ranging from focal to diffuse and strong. Adjacent benign ducts were immunoreactive in a minority of cases, similar to our previous results. Strikingly, immunoreactivity of metastatic tumors was equal to or greater than the reactivity present in the primary breast carcinoma. Carcinoma within lymphatic channels was uniformly positive for BP1 immunoreactivity. The percentage and distribution of immunoreactivity in the cohort of IBC cases were greater than a comparison cohort of non-IBC ductal carcinomas. Previous studies had revealed that BP1, a homobox gene, was expressed in 21%, 46%, and 81% of hyperplastic, *in situ*, and invasive breast lesions, respectively, while it was barely detectable in normal human breast tissues.

Another promising laboratory test has proven to be the identification of gene sequences in the tissues of IBC patients. The assays, performed by Dr. Beatriz Pogo at Mt. Sinai Hospital under a subcontract funded by this grant, are still being evaluated since Dr. Pogo wants to run the tests in triplicate before finalizing the results. The impetus for this collaborative study was provided by an earlier project which demonstrated that these sequences were far more common in Tunisian breast cancer patients than those in any other country. Since 45% of Tunisian breast cancer patients had IBC at the time of this study, we have been focusing on the patients in our IBC Registry. Until the data are finalized, it is not possible to do complete analyses on issues such as a possible correlation with disease-free survival but the preliminary data indicate that North American IBC patients express more of these sequences than non-IBC patients. The international comparison that led to this project has now been published in *Cancer* (Levine, P.H., Pogo, B.F., Klouj, A., Coronel, S., Woodson, K., Melana, S., Mourali, N., Holland, J.F. Increasing Evidence for a Human Breast Carcinoma Virus with Geographic Differences. *Cancer*. 101:721-726, 2004)

Overall clinical and epidemiologic data are still under analysis. Of the first 50 patients, 46 contacted us through the Internet and four were referred by GW physicians. Patients were diagnosed and treated in 23 different states and 2 Canadian provinces. Geographic

characteristics of patients were widespread involving rural as well as urban areas.

Eleven patients (22%) were initially treated with antibiotics up to 5 months. Four women died from IBC; two were in categories 4 and 5 and would not have been considered to have IBC by AJCC criteria. Mammograms on 70% of patients did not show any discrete mass. Sixty percent were ER+ and 38% were Her2 Neu +

Problems in accomplishing tasks

There were no apparent problems in accomplishing the tasks, although we do not have the African-American involvement we would have preferred.

Statistical test of significance

No statistical tests of significance have been performed at the current time.

Recommended changes or future work

We believe the current procedures and progress are appropriate and expect a successful outcome. We will continue on the collection and analysis of the following data:

- Molecular characterization of IBC.
- Correlation of presenting signs and symptoms, initial response to treatment, and survival.
- Molecular identification of markers of resistance to chemotherapy.
- Further characterization of risk factors for IBC.
- Collection of tissue blocks from non-IBC breast cancer patients.

KEY RESEARCH ACCOMPLISHMENTS

1. The enrollment of more than 100 patients with the smooth flow of information and biospecimens.
2. Documenting the inadequacy of current definitions of IBC and the clinical pitfalls delaying diagnosis.
3. Identification of 2 risk factors (early age at first birth and long term oral contraceptive use) for the development of aggressive breast cancer.
4. The finding of BP-1 gene in all of 45 IBC biospecimens thus far.

REPORTABLE OUTCOMES

1. Abstracts and presentations in two national breast cancer meetings (San Antonio December 2002 and 2003).
2. Inclusion of initial findings in a book chapter entitled "Breast Cancer Aggressiveness in Women of African American Descent."

CONCLUSIONS

Several important lessons have emerged from this project. First, neither the AJCC criteria for IBC or the SEER program criteria for IBC are adequate. The AJCC criteria, primarily clinical, are too extreme and miss a significant percentage of cases. The SEER criteria rely on pathologic confirmation of dermal lymphatic involvement, which is not seen in most IBC patterns. In addition, physician sensitivity to early IBC is inadequate. The high frequency of negative mammograms, the reliance on extensive use of antibiotics and delay of biopsy in a rapidly progressing cancer, and the common belief that a painful breast in a young woman "can't possibly be cancer" are examples of poor medical practice. Continued collection of data and publication in clinical and research oriented journals will hopefully lead to improved method of control.

REFERENCES

1. Levine P, Veneroso C. Eds. Williams C, Falkson C, Olopade O. Cancer Agressiveness in Women of African American Descent" in Breast Cancer in Women of African Descent." Unpublished. In Press.
2. Levine, P.H., Pogo, B.F., Klouj, A., Coronel, S., Woodson, K., Melana, S., Mourali, N., Holland, J.F. Increasing Evidence for a Human Breast Carcinoma Virus with Geographic Differences. *Cancer*. 101:721-726, 2004.